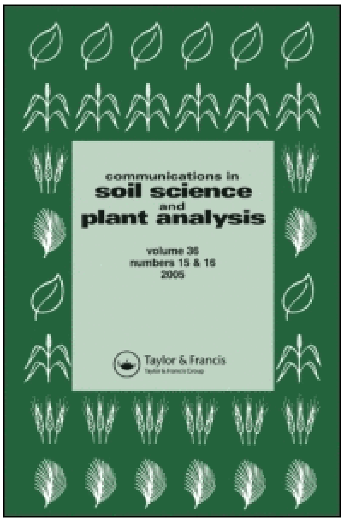

Communications in Soil Science and Plant Analysis
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597241

Incubation of Dried and Sieved Soils Can Induce Calcium Phosphate Precipitation/Adsorption

C. J. Penn [a]; R. B. Bryant [a]
[a] United States Department of Agriculture-Agricultural Research Service, Pasture Systems and Watershed Management Research Unit, University Park, Pennsylvania, USA

Online Publication Date: 01 June 2006

To cite this Article Penn, C. J. and Bryant, R. B.(2006)'Incubation of Dried and Sieved Soils Can Induce Calcium Phosphate Precipitation/Adsorption',Communications in Soil Science and Plant Analysis,37:9,1437 — 1449
To link to this Article: DOI: 10.1080/00103620600628904
URL: http://dx.doi.org/10.1080/00103620600628904

Communications in Soil Science and Plant Analysis, 37: 1437–1449, 2006

ISSN 0010-3624 print/1532-2416 online
DOI: 10.1080/00103620600628904

Incubation of Dried and Sieved Soils Can Induce Calcium Phosphate Precipitation/Adsorption

C. J. Penn and R. B. Bryant
United States Department of Agriculture-Agricultural Research Service,
Pasture Systems and Watershed Management Research Unit,
University Park, Pennsylvania, USA

Abstract: Soil incubations are a common practice typically employed in assessing the effect of some treatment on the availability and solubility of phosphorus (P). However, standard sample preparation (drying and sieving) can alter soil chemical and physical properties, resulting in possible changes in P behavior upon soil incubation. Sixty surface soil samples were collected, air dried, and sieved before being incubated at field capacity for 7 days. After incubation, soils were allowed to air dry and were analyzed along with nonincubated samples for pH and water- and Mehlich-3-extractable elements. Incubation increased pH and decreased water-soluble P, calcium (Ca), and magnesium (Mg) relative to nonincubated soils. Increases in pH may have been due to increased solubility of residual calcium carbonates by drying and sieving. This increase in pH among soils with sufficient levels of P, Ca, and Mg resulted in the formation of Ca and Mg phosphates as confirmed by chemical speciation modeling.

Keywords: Incubations, phosphorus solubility

INTRODUCTION

Soil incubations are a common, inexpensive, and simple method typically used in assessing the effect of some treatment on a soil property or crop response. Treatments often consist of nutrient and organic matter

Received 10 May 2005, Accepted 16 September 2005
Address correspondence to C. J. Penn, Department of Plant and Soil Sciences, 368 Agricultural Hall, Oklahoma State University, Stillwater, OK 74078, USA. E-mail: chad.penn@okstate.edu

amendments, metals, various waste products, pesticides, or other organic chemicals. Incubations are also conducted to test various conditions such as pH, redox, moisture, temperature, and atmospheric composition. Soil incubations are especially common among researchers who conduct studies on phosphorus (P). Accurate determination of soil P concentrations is important to soil testing for both agronomic and environmental concerns because proper P management for preventing nonpoint losses of P in runoff and maximizing crop growth are dependent upon accurate soil P measurements of field samples as well as soils used in incubation experiments.

A potential problem in conducting incubation studies intended to address P availability or the effect of some treatment on P availability is that soil preparation (drying, grinding, sieving) for such studies may alter the chemistry of the system upon rewetting. A change in soil chemistry (for example, pH, mineral solubility, ionic strength, surface charge characteristics) could significantly influence P solubility and availability (among other things).

Sieving destroys soil structure and alters the size and distribution of pore spaces, which has been shown to stimulate microbial activity (Powlson 1980; Shah, Adams, and Haven, 1990), alter water retention properties (Unger 1975), and increase the surface area for sorption processes to take place (Hantschel et al. 1988). However, Chapman et al. (1997) showed that total dissolved P of field moist soils was not significantly different from sieved field moist soils after storage for more than 1 day at 4°C. Bartlett and James (1980) found that water-extractable P, calcium (Ca), and magnesium (Mg) increased in dried and sieved soils relative to field moist soils. As a result, these potential changes could interfere with treatment effects and/or control (nonamended soil) treatments upon rewetting, both of which would result in misinterpretations of data. This last point is important because many incubation experiments typically involve using dried and sieved soils that are moistened up upon initiation of the study. For whatever reason, control (unamended) soil P levels will typically decrease at the onset of an incubation study (Maguire et al. 2001; Leytem, Sims, and Coale 2004; Kalbasi and Karthikeyan 2004; Siddique and Robinson 2004).

The objective of this study was to examine the effect of rewetting (incubation) on soil water-soluble P (WS-P) and Mehlich-3 P (M3-P) concentrations and determine why any changes occurred.

MATERIALS AND METHODS

Site Management and Soil Collection

The soils used in this study were taken from an alfalfa (*Medicago sativa*), hay, and two pasture (pasture 1 and 2) fields located on two dairy farms in Delaware County, New York, in the Catskill mountain area. The hay field consisted of an orchard grass (*Dactylis glomerata*), white clover (*Trifolium repens*), and red clover (*Trifolium pratense*) mix. Pasture 1 and 2 consisted of the same set of mixed grasses but was dominated by orchard grass. Prior to when soil samples were taken (2001), the alfalfa field was in corn (*Zea mays*) production. Each field was extensively sampled by taking 100 soil core samples (0–5 cm) on a 10-m grid. Soil samples were air dried, ground, and sieved with a number 10 sieve (2-mm mesh opening) before analysis.

All four fields were routinely applied with dairy manure and lime. The alfalfa, pasture 2, and hay fields were located on a Lewbeach silt loam (coarse loamy, mixed, semiactive, frigid Typic Fragiudepts), and half of the hay field also consisted of a Willowemoc silt loam (coarse loamy, mixed, semiactive, frigid Typic Fragiudepts). Willowemoc is considered moderately well drained, whereas the Lewbeach soil is well drained. Pasture 1 was located on a Lewbeach soil.

Soil Sample Preparation and Chemical Analysis

Twenty-five grams of soil from 15 soil samples from each site were incubated at 25°C and 30% moisture (by weight) in a glass jar for 7 days. After day 7, incubated samples were air dried and homogenized before chemical analysis.

The individual soil samples (100 from each field) and the incubated soil samples were extracted with Mehlich-3 (M3) and water (WS) for P. M3 extractions were conducted by shaking 2 g of air-dried soil with 20 ml of M3 solution (0.2 M $CH_3COOH + 0.25$ M $NH_4NO_3 + 0.015$ M $NH_4F + 0.13$ M $HNO_3 + 0.001$ M EDTA) end over end for 5 min followed by filtration with Whatman #1 paper (Mehlich 1984). All solutions were analyzed for P, and the samples used in the incubation were additionally analyzed for aluminum (Al), iron (Fe), Ca, and Mg by inductively coupled plasma-atomic emission spectrometry. Water extractions were conducted by shaking 2 g of air-dried soil with 20 mL of deionized water end over end for 1 h, followed by centrifuging (2,500 rpm at 5 min) and filtering with 0.45-μm membranes. All extracts were analyzed for P, and soils used in the incubation were additionally analyzed for Ca, Mg, Al, and Fe by ICP-AES and $NO3^-$, NH_4^+, SiO_4^-, Br^-, Cl^-, and SO_4^{2-} colorimetrically by flow injection analysis (Lachat Instruments, method numbers 10-107-04-1-A, 10-107-06-1-B, 10-114-27-1-A, 10-135-21-2-B, 10-117-07-1-B, and 10-116-10-1-A, respectively). The pH and ionic strength (as estimated by electrical conductivity [Griffin and Jurinak 1973]) were also measured on each water extract from incubated soils. Soil pH was measured by using a glass electrode and a 1 : 1 ratio of soil–deionized water. The data from the water extracts were used to predict the presence of solid mineral phases as determined by MINTEQA2 speciation model (Allison, Brown, and Novo-Gradac 1991).

Statistical Analysis

Analysis of variance was used to determine the least significant difference between means at $P < 0.05$. All means, median, range, correlation, and analysis of variance procedures were determined using the standard procedures of SAS (SAS Institute 1998). Means from incubated and nonincubated soils were tested for significant differences using a paired t-test assuming unequal variances.

RESULTS AND DISCUSSION

Background Mehlich-3 Phosphorus, Aluminum, Iron, Calcium, and Magnesium

WS P concentrations were similar for all four fields, whereas M3 P concentrations were lower for the pasture 1 and hay fields as compared to pasture 2 and alfalfa fields (Table 1). These differences in M3 P among soils similar in WSP suggest that there may be differences in P forms between pasture 1 and hay, and pasture 2 and alfalfa. Based on the Pennsylvania State University soil testing guidelines (Beegle 2002), the mean M3 P concentrations for each site are considered "above optimum" ($>50\,\mathrm{mg\,P\,kg^{-1}}$) in regard to agronomic P needs. As expected, M3 Al was higher for alfalfa and hay fields compared to the two pasture sites (Table 1), likely due to the more recent tillage of the alfalfa and hay fields that resulted in more subsoil (which is typically high in Al relative to topsoil) being mixed with the topsoil sampled. The same trend was not observed with M3 Fe, perhaps because Mehlich 3 is much more efficient at extracting soil Al than Fe. M3 Mg and particularly M3 Ca were greater for pasture 1 and hay as compared to pasture 2 and alfalfa. This may be an effect of a more recent or greater lime application to pasture 1 and hay fields.

Unexpectedly, three of the four fields had M3 P concentrations significantly correlated ($p < 0.05$) with M3 Mg (r^2 values of 0.53, 0.77, and 0.44 for pasture 1, pasture 2, and alfalfa, respectively). In addition, the alfalfa field showed a significant correlation ($p < 0.05$) between M3 P and M3 Ca (r^2 was 0.57). Similarly, Josan et al. (2005) found that WS Mg was well correlated to WS P among dairy-impacted soils. In their study using a chemical speciation model, analysis of soil extracts and leachates from active and abandoned dairy soils resulted in the prediction of solid Ca and Mg phosphates for more than 30% of the active dairy soils. The significant M3 P correlations with Mg and Ca in this study suggest the possibility of the presence of solid Mg and Ca phosphates or simple co-correlations due to dairy manure applications and the animals' diet or the recent lime application history. According to a study by Kalbasi and Karthikeyan (2004), dairy manure had Ca-to-P and Mg-to-P molar ratios of 2.73 and 2.85. Nair, Graetz, and

Table 1. Mean, median, and range (mg kg^{-1}) for soil extractable P, Al, Fe, Ca, and Mg among the 100 individual samples taken from each field at a depth of 0–5 cm

Soil parameter	Pasture 1			Pasture 2			Alfalfa			Hay		
	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
WS P[a,b]	12	10	2–34	18	16	3–125	16	15	5–36	15	16	6–36
M3 P[c]	157	158	46–346	138	135	39–586	206	204	124–366	186	179	113–279
M3 Al	611	613	265–895	553	535	352–891	732	740	548–909	699	704	259–1025
M3 Fe	348	366	178–482	166	163	87–251	153	138	94–276	291	291	136–427
M3 Ca	2244	2309	774–3859	1679	1684	984–2122	1556	1563	1185–2222	2366	2294	1617–3746
M3 Mg	343	343	163–924	258	253	144–688	212	209	161–477	273	253	168–524

[a]Least significant difference at $p = 0.05$ for WSP, Mehlich 3 P, Al, Fe, Ca, and Mg across sites is 2.57, 14, 31, 15, 110, and 22, respectively.
[b]Water-soluble P.
[c]Mehlich-3.

Portier (1995) found that Ca- and Mg-associated P was the dominant soil P fraction (approximately 70% of total P) among active and abandoned dairy systems in South Florida's Lake Okeechobee watershed. In their study, P forms were estimated using a chemical fractionation method.

Effects of Incubation on Soil Properties

Incubation of the soil samples representing each field resulted in dramatic changes in certain soil properties. Table 2 indicates that for every site, pH of the incubated samples was greater than their dry counterparts. Mortland and Raman (1968) hypothesized that as a soil is dried, the cations more strongly polarize the remaining water molecules, making them more acidic than the free water. However, less is known about the effect on pH of rewetting dried soils. The exact reason for the increase in pH is not known, but it is likely that the drying, grinding, and sieving of the individual soil samples caused an increase in the surface area for each sample as well as exposed any residual calcium carbonate (from previous lime applications), which then dissolved under the ideal temperature and moisture conditions of the incubation. Ideally, this continued dissolution of residual calcium carbonate would also occur in the field but at a much slower rate because of the conditions. Also, the natural process of acidification in the agricultural environment would likely counteract any potential for the residual calcium carbonates to increase pH as much as the incubated sieved soils did in this study (at least a 1-unit increase in pH for many samples). One potential consequence of the increased pH among incubated soils is possible effects on P forms and solubility.

Incubation of soil samples also appeared to have a significant effect on WS P (Table 2). Soil samples that were incubated had significantly less WS P than the dry samples not incubated. One possibility for the decrease in WS P with incubation could be microbial uptake of P into forms not soluble in water caused by increased microbial activity. The increase in microbial activity in this case would be a result of ideal conditions (temperature and moisture) as well as an increase in pH to more neutral levels (Table 2). For example, McDowell and Sharpley (2003) showed that the microbial P pool was rather large and dynamic in a simulated stream environment (fluvarium), accounting for 34 to 43% of sediment P uptake from manure-rich overland flow. The possibility of microbial P uptake during the incubation cannot be discounted because these soils have a history of receiving dairy manure, which adds significant amounts of dissolved organic carbon and various cations, promoting microbial activity.

A more likely reason for the appreciable decrease in WS P with incubation is the shift of labile inorganic P forms into less water-soluble inorganic P forms as influenced by pH. In this case, because incubation increased pH, the inorganic P form most likely to increase would be Ca-associated P.

Table 2. Comparison of the average pH, water-soluble (WS), and Mehlich-3 (M3)-extractable P, Ca, Mg, Al, and Fe ($mg\,kg^{-1}$) between nonincubated and incubated samples from each site

Soil parameter	Pasture 1		Pasture 2		Alfalfa		Hay	
	Preincubate	Postincubate	Preincubate	Postincubate	Preincubate	Postincubate	Preincubate	Postincubate
WS P	46.0**	28.4	46.4**	25.4	21.8**	15.7	48.0**	34.6
WS Ca	218.9**	96.1	113.1	43.5	81.7**	32.2	181.6**	66.1
WS Mg	50.7**	21.0	30.1**	12.7	21.8**	8.7	37.6**	13.4
WS Al	15**	19.8	22.2**	37.1	20.8**	27.1	19.3*	23.5
WS Fe	10.8	12.0	15.2**	22.5	15.6**	20.1	15.3	16.3
M3 P[a]	204	220	183**	205	263	238	232	251
M3 Ca	3027	2776	1897	1897	1804**	1538	2784	2821
M3 Mg	393	384	295	293	250*	230	328	339
M3 Al	623*	526	671	651	937**	866	719*	677
M3 Fe	305	263	251	253	237	246	310	274
pH	6.22*	6.98	5.78**	6.73	5.94**	6.91	6.08**	7

Note: **, * Indicates significant differences between pre- and postincubated soils at $p = 0.01$ and 0.05, respectively.
[a]Mehlich-3.

In contrast to WS P, incubation tended to cause a general increase in M3 P for three of the four sites but was only statistically significant for pasture 2 (Table 2). Because Mehlich-3 is a much stronger soil extractant compared to water, it is not as sensitive in detecting small changes in P solubility. From Table 2 it appears as if incubation of the soil samples resulted in a shift of P into forms that are less soluble in water, yet more soluble in acid (Mehlich 3) compared to preincubated soils. Again, this suggests that soil P may be shifting to less water-soluble inorganic P forms such as Ca and Mg phosphates. Evidence for this is supported by the fact that Ca and Mg phosphates are highly soluble in acidic extractants such as Mehlich 3 and because P tends to precipitate/adsorb onto Ca and Mg with increases in pH (Lindsay 1979), as Table 2 indicates that pH clearly increased with incubation.

As expected then, incubation of the soils also resulted in a decrease in WS Ca and Mg; therefore as the soils were incubated and the pH increased (Table 1), labile P, Mg, and Ca were removed from solution (as indicated by decreases in WS P, WS Mg, and WS Ca) because of Ca and Mg phosphate precipitation/adsorption reactions. However, these newly formed Ca and Mg phosphates are very soluble in an acid extractant such as M3 (Table 1). Curtin and Syers (2001) also showed that during incubation, decreases in soil WS P were accompanied with additions of lime, attributed to P precipitation or coadsorption with Ca and Mg.

Similarly, Sharply et al. (2004), determined that although soil WS P increased with M3 P, the percentage of M3 P as WS P decreased with increasing M3 P concentrations. The authors attributed this to the buildup of Ca phosphates in the soil (i.e., the acid M3 solution extracts a disproportionately high amount of Ca phosphates relative to WS P). This was evident over a diverse group of soils (20 sites representing soils from Pennsylvania, New York, and Oklahoma) previously applied with dairy, poultry, or swine manure. Other studies have also shown that increased Ca can influence P dynamics. For example, Siddique and Robinson (2004) amended soils with poultry litter, cattle slurry, biosolids, or potassium phosphate on a P-equivalent basis ($100\,\mathrm{mg\,P\,kg^{-1}}$) to examine changes in P sorption and kinetics of P release. The authors found that the rate constant describing the rate of P release to solution was positively correlated with soil exchangeable Ca (i.e., the rate of P desorption decreased with increasing exchangeable Ca). In this study, it is likely that exchangeable Ca and Mg were increased as a result of drying, grinding, and sieving the soils before being consumed by precipitation/adsorption with P during incubation as influenced by the increase in pH.

In addition, note in Table 2 that WS Al and Fe increased with incubation. This increase was unexpected because solution Al and Fe typically decrease with increases in pH due to the formation of metal hydroxides. This observed decrease in WS Al and Fe may be an indication that P adsorbed/precipitated with Al and Fe is shifting to Ca, typical for soils that increase in pH from $\sim$5.5 to $\sim$7.3 (Lindsay 1979). Thus, the initial shift in P from Al and Fe to Ca and Mg would result in a temporary increase in WS Al and Fe until the insoluble metal hydroxides are formed as the soil approaches equilibrium. Another hypothesis is that the increase in pH caused an increase in soluble organic species that could complex Al and Fe. On the other hand, M3 Al concentrations decreased with incubation, indicating that the larger pool of soil Al has actually become less soluble in an acidic solution.

It is important to note, however, that for this study we are in no way implying that the soil P dynamics are dominated by Ca phosphates, only that Ca phosphates are forming as a result of incubating previously limed/dairy manure applied soils that were dried, ground, and sieved prior to incubation. To determine if Ca and Mg phosphates were only present in the soils after incubation, the MINTEQA2 chemical speciation program was used to model the soils both before and after incubation. Results indicated that the only precipitated phosphate species predicted in any case was hydroxyapatite ($Ca_5[PO_4]_3OH$). When soils from all sites were grouped together, incubation of the dried and sieved soils resulted in a 32% increase in the presence of hydroxyapatite. The formation of hydroxyapatite upon rewetting (incubation) of the soils is illustrated in Figure 1. Points lying above the theoretical hydroxyapatite line indicate oversaturation with

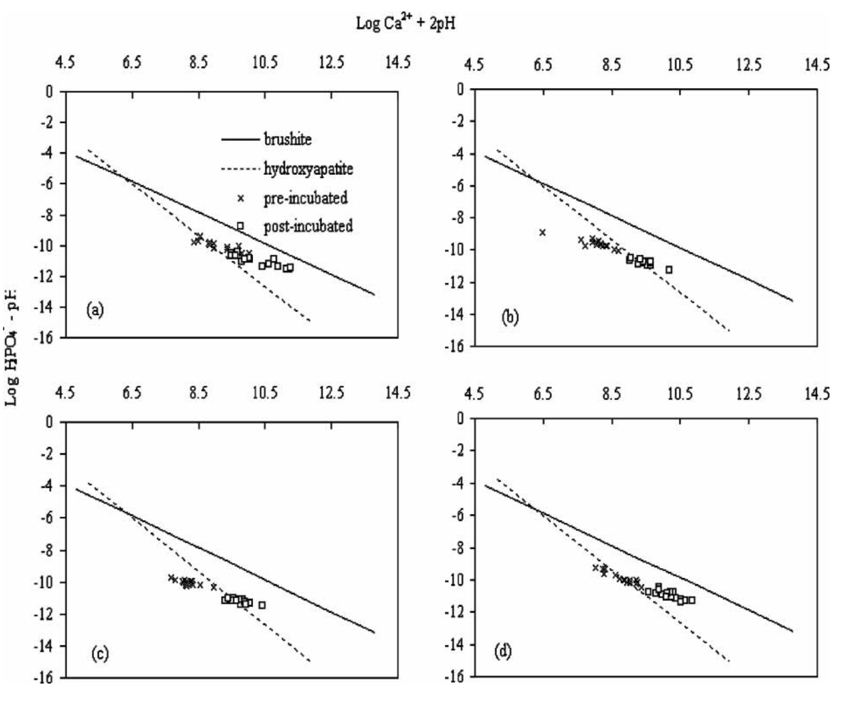

Figure 1. Solubility diagrams plotting pre- and postincubated soils from pasture 1 (a), pasture 2 (b), alfalfa (c), and hay (d) in comparison to theoretical brushite ($CaHPO_4$ $2H_20$) and hydroxyapatite ($Ca_5[PO_4]_3OH$) (Lindsay 1979).

respect to that mineral, whereas points falling below the line indicate under-saturation. Note that although none of the soils appeared to precipitate the more highly soluble brushite ($CaHPO_4 \cdot 2H_2O$) mineral, incubation of the soils tended to push the points from below to above the theoretical hydroxyapatite line, indicating possible formation of that mineral.

Implications for Soil Testing and Assessing Potential Phosphorus Losses in Runoff

Changes in soil WS P and M3 P can have significant environmental implications because both are often used in P-loss prediction models and assessment tools because each are typically well correlated with dissolved P or total P in runoff (Torbert et al. 2002; Penn, Mullins, and Zelazny 2005; Sharpley, McDowell, and Kleinman 2001). In this case, because the WS P concentrations were lower than the nonincubated soils, interpretation of the results for purposes such as assessing potential losses of dissolved P in runoff would result in an underprediction. On the other hand, using M3 P to assess potential dissolved and total P losses would result in an overprediction because the incubation tended to increase M3 P. Similar potential problems would also occur in such a case if M3 P was being used to make P application recommendations for crops. However, all of these possible misinterpretations depend on the act of measuring soil P after incubating soils that were previously dried, ground, and sieved; this is not a standard practice for soil testing in regard to making crop P recommendations or assessing potential P losses. A more important issue is that this process of Ca-P formation coupled with WS P reduction could potentially occur in the field.

In regard to agricultural practices and natural soil processes, a likely scenario for Ca-P formation would be from continuous application of manure or waste products high in Ca and potentially capable of increasing soil pH. However, some products may also add P to the soil and therefore not decrease WS P. For example, Sharpley, McDowell, or Kleinman (2004) showed that soils with a history of receiving dairy, poultry, or swine manure contained a greater amount of Ca-P compared to their unamended counterparts as indicated by both chemical P fractionation and chemical speciation modeling. However, the authors stated that a consequence of using M3 P in assessing potential dissolved P losses among soils dominated with Ca-P is an overprediction of potential P losses because M3 extracts a disproportionate amount of Ca-P relative to Ca-P soluble in runoff water.

Another possible field scenario that would result in Ca-P formation but instead potentially decrease soil WS P (as opposed to the application of waste products high in Ca and P), would be the practice of lime application intended for increasing soil pH. Theoretically, lime applications that increase soil pH can have several effects on P sorption. Increases in pH will influence the surface charge characteristics of a soil to become more negative (discouraging P adsorption) but also cause some solution P in the form $H_2PO_4^-$ to shift to the more negatively charged HPO_4^{2-}, which is the P species considered to be adsorbed (Barrow 1984). In addition, the increased soil solution ionic strength and presence of divalent cations (Ca and Mg) among soils in which the pH is above the point of zero change will also promote increased negative surface charge. The degree in which each of these occurs is largely dependent upon soil properties, mainly mineralogy. Another possible effect of liming acid soils is the precipitation of Ca-P (Naidu et al. 1990). Although this study was not intended to investigate the effects of soil lime application on P solubility, the results suggest that in some cases lime application could possibly decrease soil WS P by precipitating P with Ca. Similarly, Curtin and Syers (2001) found that soils amended with $CaCO_3$ had depressed levels of WS P compared to the unamended control soil. In their study, six different New Zealand soils with an initial pH of 5.1 to 5.5 were incubated with four rates of $CaCO_3$ to raise pH incrementally up to 6.5 and also received inorganic P (KH_2PO_4) at three different levels. The authors noted that P addition appeared to induce a decrease in soluble Ca and Mg concentrations, attributed to either coadsorption or precipitation of Ca and Mg with P.

Decreases in soil WS P among high-P soils already abundant in Ca and Mg (such as those used in this study) through lime application represent a relatively inexpensive, easy, and acceptable method for potentially controlling dissolved P losses in runoff from acid, noncalcareous soils. However, further research is necessary to determine if lime-induced WS P decreases translate into decreases in runoff dissolved P concentrations. As opposed to runoff dissolved P concentrations, lime applications would likely not reduce total P concentrations in runoff unless the increases in soil Ca, Mg, pH, and ionic strength cause a decrease in soil erosion through significantly improving soil physical properties such as aggregation. Again, the potential decrease in soil erosion through improved soil physical properties will likely depend on soil properties such as mineralogy.

CONCLUSIONS

Incubation (rewetting) of previously dried and sieved soil samples altered the chemistry of the samples relative to nonincubated soils. Particularly, incubation caused soil pH to increase nearly 1 unit for most samples. Although the exact reason for the pH increase is not known, we speculate that drying, grinding, and sieving the individual samples (which had a history of lime application) increased the surface area of the soil and further exposed any residual calcium carbonate. The ideal temperature and moisture conditions of the incubation then caused the residual calcium carbonate to further dissolve.

This increase in pH combined with a sufficient Ca source then caused a decrease in soil WS P due to Ca precipitation with P. As a result of soil P associated with Al and Fe pools shifting to P precipitated/adsorbed with

Ca, M3 P concentrations increased in the majority of the incubated composite samples because Ca-P is soluble in acid solutions. As expected, WS Ca and Mg decreased with incubation for nearly every composite sample because of precipitation/adsorption with P. Chemical speciation modeling indicated that incubation of the soils resulted in a 32% increase in the number of samples containing hydroxyapatite.

Application of the results of this study to soil testing agricultural sites for agronomic or environmental purposes may be limited because it is not standard practice to rewet dried and sieved subsamples before extracting. Instead, these results are more important in regard to incubation studies intended to measure effects of some treatment on soil P because it is common practice to air dry and sieve soils before use in such a study. If the soil has significant concentrations of Ca and P and also possesses residual calcium carbonate (from a previous lime application), then incubation of the soils could result in a shift of P into Ca-P, which could interfere with treatment effects and/or cause a misinterpretation of the data.

The results of this study also imply that among acidic field soils with high levels of M3 P, WS P, Mg and Ca, increases in soil Ca and pH through lime application could cause a reduction in WS P through P precipitation with Ca. Such decreases in soil WS P could potentially help to reduce nonpoint losses of dissolved P in runoff because soil WS P is widely considered an indicator for potential dissolved P loss concentrations. However, this is only speculation, and more research on that particular topic is necessary to confirm such a hypothesis.

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